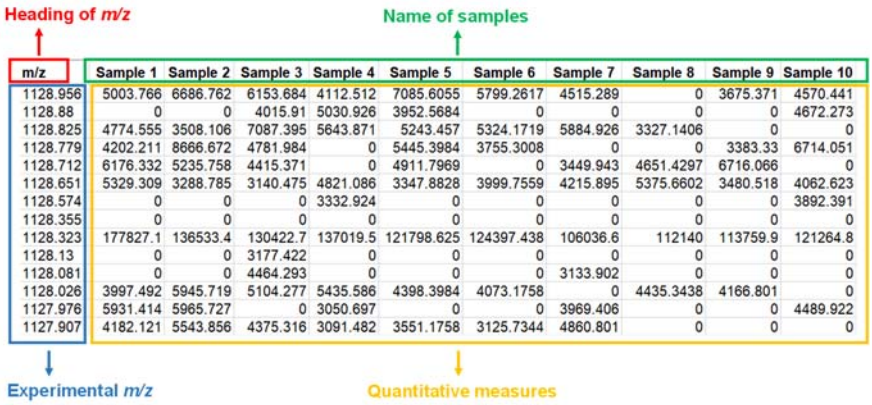
ReadMe file for LipidQuant 1.0

Basic instructions how to use LipidQuant 1.0 for automated data processing in lipid class separation - mass spectrometry quantitative workflows (updated June 4, 2021)

Input data to LipidQuant 1.0

1. It has to be **txt format** or **Excel sheet** including all *m/z* features in the first column with the heading of *m/z* followed by individual samples containing the intensities or other quantitative measures for each *m/z* feature (**Figure 1**).

Figure 1. Example of an input table to the LipidQuant 1.0.



m/z	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
1128.956	5003.766	6686.762	6153.684	4112.512	7085.6055	5799.2617	4515.289	0	3675.371	4570.441
1128.88	0	0	4015.91	5030.926	3952.5684	0	0	0	0	4672.273
1128.825	4774.555	3508.106	7087.395	5643.871	5243.457	5324.1719	5884.926	3327.1406	0	0
1128.779	4202.211	8666.672	4781.984	0	5445.3984	3755.3008	0	0	3383.33	6714.051
1128.712	6176.332	5235.758	4415.371	0	4911.7969	0	3449.943	4651.4297	6716.066	0
1128.651	5329.309	3288.785	3140.475	4821.086	3347.8828	3999.7559	4215.895	5375.6602	3480.518	4062.623
1128.574	0	0	0	3332.924	0	0	0	0	0	3892.391
1128.355	0	0	0	0	0	0	0	0	0	0
1128.323	177827.1	136533.4	130422.7	137019.5	121798.625	124397.438	106036.6	112140	113759.9	121264.8
1128.13	0	0	3177.422	0	0	0	0	0	0	0
1128.081	0	0	4464.293	0	0	0	3133.902	0	0	0
1128.026	3997.492	5945.719	5104.277	5435.586	4398.3984	4073.1758	0	4435.3438	4166.801	0
1127.976	5931.414	5965.727	0	3050.697	0	0	3969.406	0	0	4489.922
1127.907	4182.121	5543.856	4375.316	3091.482	3551.1758	3125.7344	4860.801	0	0	0

2. Lipid class separation: one txt file = one lipid class.

Attention!

- One txt file can be used for more lipid classes due to the same or almost the same elution window, e.g., SM + LPC or DG and Chol maybe included in one file. Make sure that there are no mass interferences between two lipid classes in one txt file.
- Individual columns in txt format have to be separated by a tabulator, but not comma or dot.
- Decimal point (for *m/z* values and quantitative measures) has to be used, but not comma.

LipidQuant 1.0

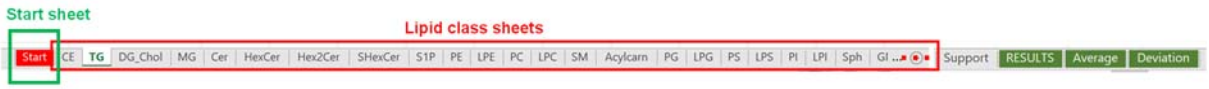
1. Open the LipidQuant 1.0.

Attention!

- Excel Macro has to be activated.

2. Go to the Start sheet (**Figure 2**), press the button "Clear all concentrations" to be sure that all data sheets are empty for starting a new processing.

Figure 2. Sheets of LipidQuant 1.0.



3. Set the concentration of internal standards and ***m/z* tolerance window** in all sheets of lipid classes, which you want to quantify (**Figure 3**).

Attention!

- You can set a maximum of 3 lipid standards within one lipid class.
- You have to define the order of the IS in the database (in cells C3, C4, or C5). Count the number of lines starting from line 10 until the IS is written (**Figure 4**).

Figure 3. Example of TG lipid class sheet with given IS information (annotation, order in the database, *m/z*, and concentration) and *m/z* tolerance window. The same structure is used for each lipid class sheet.

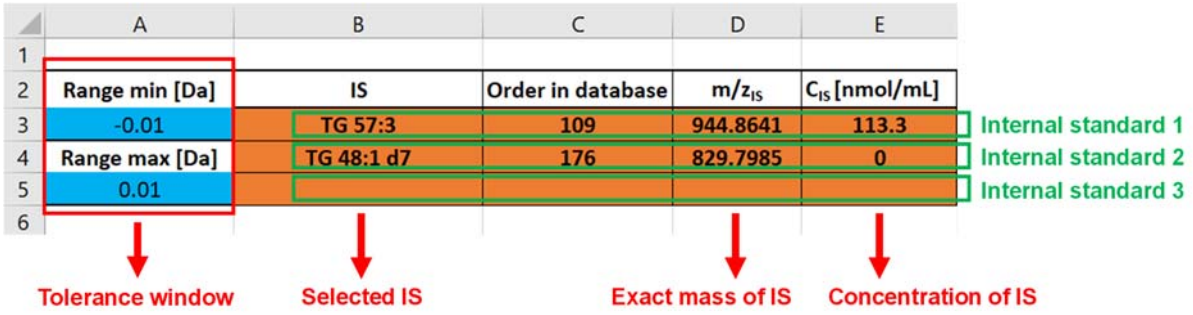
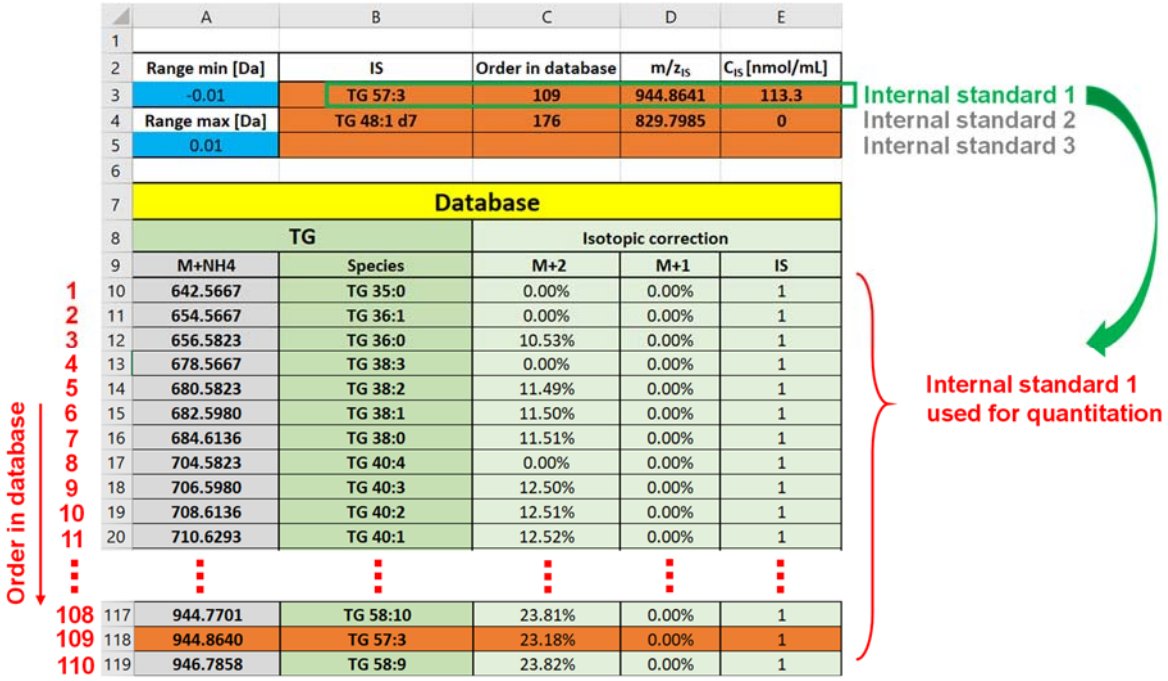


Figure 4. Definition of selected IS for quantitation. The same structure is used for each lipid class sheet.



4. Define the internal standard (IS), which should be applied for the quantitation of lipid species by setting the IS number 1, 2, or 3 to the lipid class database (column E) for all lipid classes, you want to quantify (**Figure 4**).

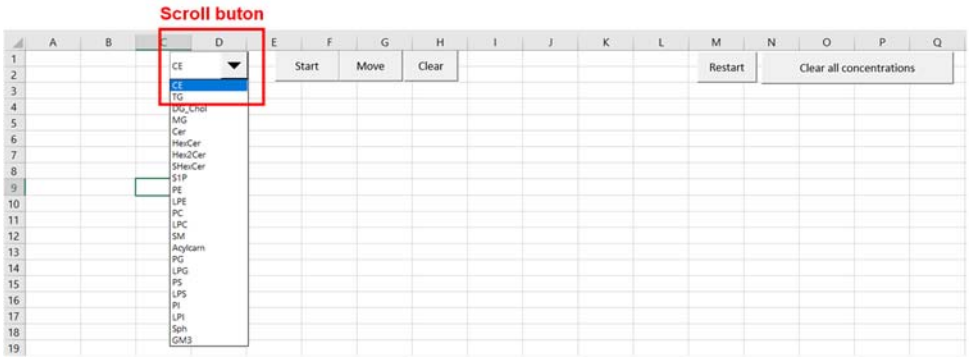
Attention!

- If you use the internal standard 2 or 3, you have to set number 2 or 3, respectively, to the database.

5. Go to the Start sheet of LipidQuant 1.0.

6. Choose the lipid class, which you want to quantify using the scroll button (**Figure 5**), open the input table of this class, select all, and copy the content into LipidQuant 1.0 by pasting in cell A1.

Figure 5. Start sheet of LipidQuant.



7. Press Start button. Now the LipidQuant 1.0 is comparing the exact *m/z* with the experimental *m/z* value according to the applied *m/z* tolerance.

8. When the processing is finished, a colored table appears. You can remove all lines, which are not green (**Figure 6**), as this lipid species are not within the tolerance, or follow subsequent instructions.

Attention!

- The number in the yellow column E illustrates only the position of the species in the database (class sheet).
- Light yellow highlighted lipid species (column C in **Figure 6**) are within two times the mass tolerance. When you decide to anyhow keep and quantify these lipid species, you have to put number 1 to the cell in column C and add the number of position of lipid species (cell in column E) in the database (**Figure 7**).
- Red marked lipid species (column C in **Figure 5**) show more detected lipids within the tolerance range. Remove all of them or choose the one you want to quantify and remove the second one (**Figure 8**).

Figure 6. Example of the colored table.

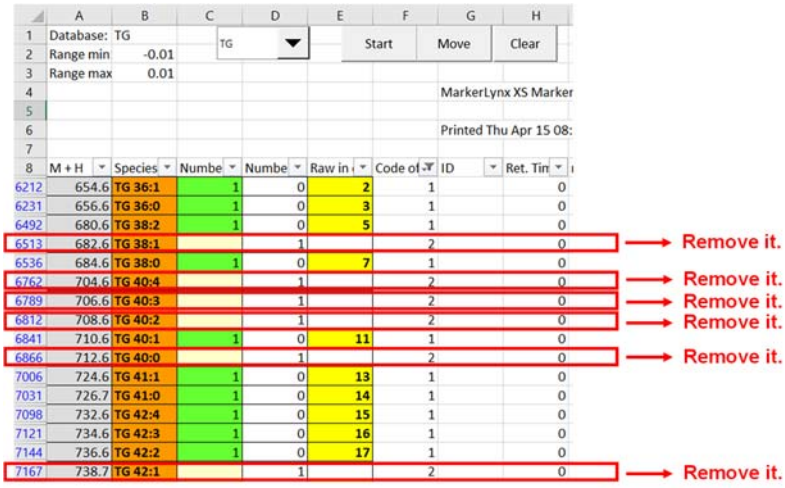


Figure 7. Example of changes in a colored table.

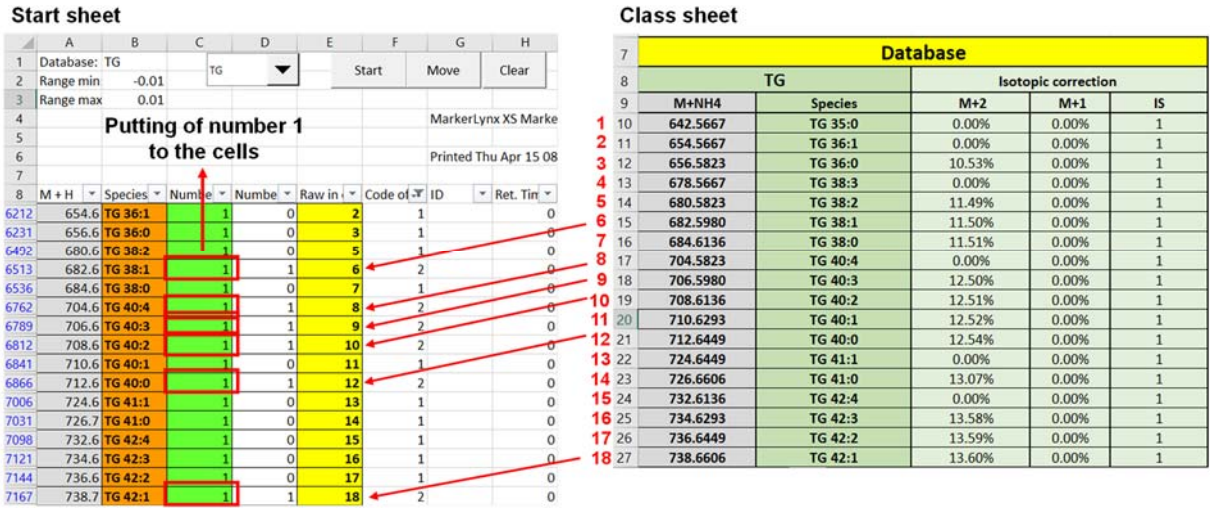
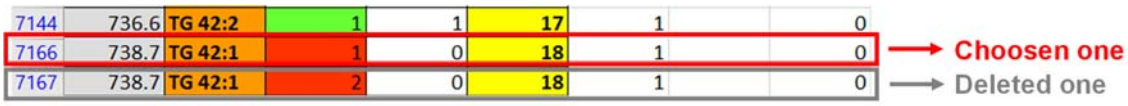


Figure 8. Example of red marked lipid species.



9. After removing or changing of some lines (cells in columns C and E), **press Move button**. Now the LipidQuant 1.0 performs the isotopic correction, quantitation and moves the results to the class sheet.

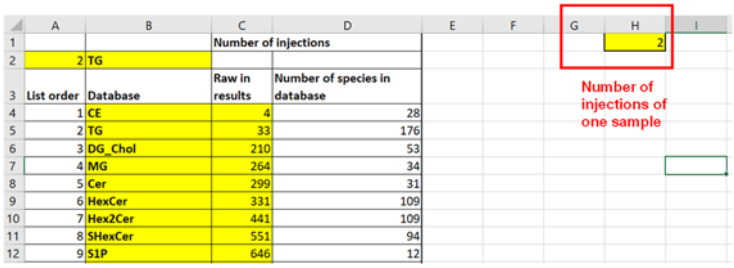
10. Once it is finished, a window appears with "Finish", **press OK.**

11. Press Clear button (in Start sheet) and continue with the next lipid class according to items 6 – 10 until you process all lipid classes for quantitation.

12. When you make multiple injections of one sample, set the number of injections (cell H1) in Support sheet **(Figure 9**).

13. Go to the Result sheet and press Insert data. This may take longer time. You will get a summary table of your lipid species concentration in all samples.

Figure 9. Support sheet of LipidQuant.



	A	B	C	D	E	F	G	H	I
1			Number of injections					2	
2	2	TG							
3	List order	Database	Raw in results	Number of species in database					
4	1	CE	4	28					
5	2	TG	33	176					
6	3	DG_Chol	210	53					
7	4	MG	264	34					
8	5	Cer	299	31					
9	6	HexCer	331	109					
10	7	Hex2Cer	441	109					
11	8	SHexCer	551	94					
12	9	S1P	646	12					

Attention:

- The number of injections in Support sheet has to be set before you insert data to the Result sheet. Do not forget save changes.

14. Average and deviation of lipid species concentrations for multiple injections will be shown in the summary table in Average and Deviation sheets, respectively.

Attention!

- Multiple injections of one sample have to be in subsequent lines without any interruption.
- Average and deviation values in the corresponding sheets will be saved according to the name of the first injection of sample.

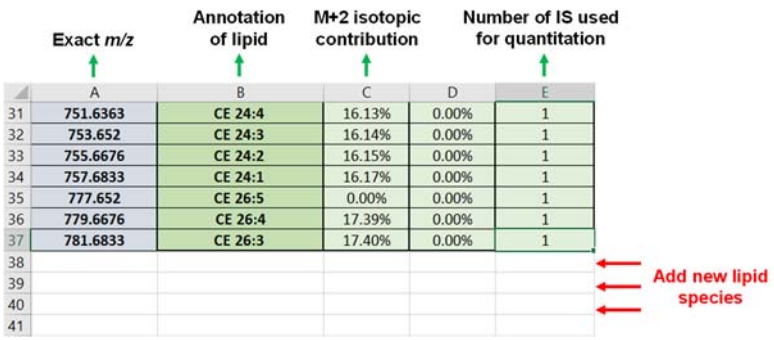
Modification of LipidQuant 1.0

Addition of more lipid species into the existing lipid class sheet

1. Open the lipid class sheet, which you want to modify.

2. Add lipid species including exact *m/z*, annotation of lipid, M+2 isotopic contribution of lipid, and the number of IS used for quantitation to the end of the lipid database (**Figure 11**).

Figure 10. Addition of new lipid species into the existing lipid class sheet.

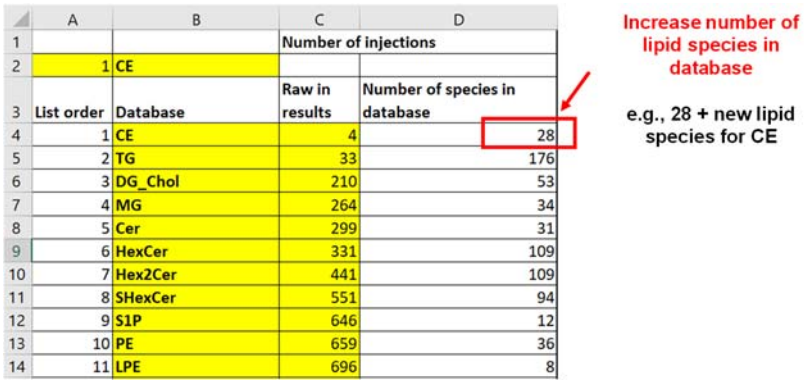


	A	B	C	D	E
31	751.6363	CE 24:4	16.13%	0.00%	1
32	753.652	CE 24:3	16.14%	0.00%	1
33	755.6676	CE 24:2	16.15%	0.00%	1
34	757.6833	CE 24:1	16.17%	0.00%	1
35	777.652	CE 26:5	0.00%	0.00%	1
36	779.6676	CE 26:4	17.39%	0.00%	1
37	781.6833	CE 26:3	17.40%	0.00%	1
38					
39					
40					
41					

3. Go to the Support sheet to the LipidQuant 1.0.

4. Increase the number of lipid species in the database within the lipid class, which you want to modify (**Figure 12**).

Figure 11. Support sheet.



	A	B	C	D
1			Number of injections	
2	1	CE		
3	List order	Database	Raw in results	Number of species in database
4	1	CE	4	28
5	2	TG	33	176
6	3	DG_Chol	210	53
7	4	MG	264	34
8	5	Cer	299	31
9	6	HexCer	331	109
10	7	Hex2Cer	441	109
11	8	SHexCer	551	94
12	9	S1P	646	12
13	10	PE	659	36
14	11	LPE	696	8

5. Save the changes.

6. Process data in the same way, as described above.

Addition of new lipid class

1. Create a new lipid class sheet according to the existing one, which can be used as a template.

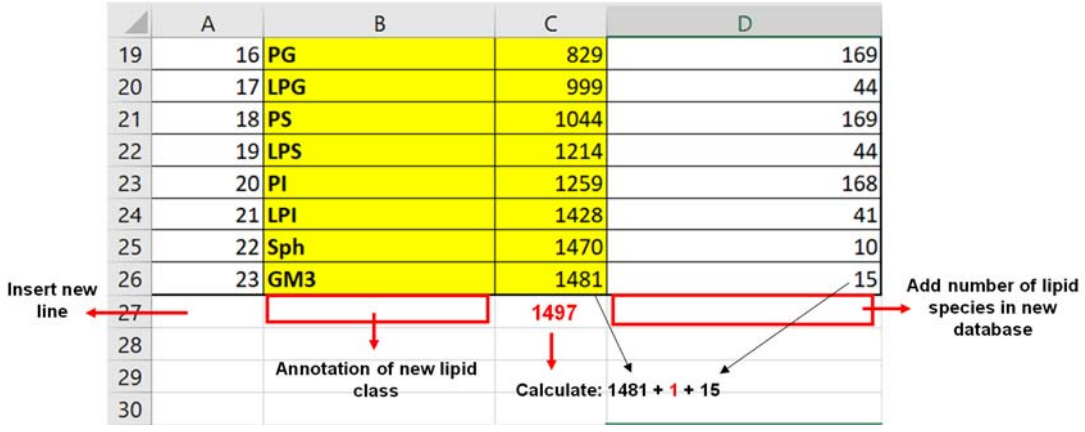
2. Add lipid species of the new created lipid class to the database including exact *m/z*, annotation, M+2 isotopic contribution, and number of IS used for their quantitation.

3. Add information about the used IS (annotation, m/z, the order in database, and concentration).

4. Go to the Support sheet.

5. Insert the new line, add the annotation of new lipid class (column B), the number of lipid species in the database (column D), and calculate the number of lines in results (column C) (**Figure 13**).

Figure 12. Support sheet.



	A	B	C	D
19	16	PG	829	169
20	17	LPG	999	44
21	18	PS	1044	169
22	19	LPS	1214	44
23	20	PI	1259	168
24	21	LPI	1428	41
25	22	Sph	1470	10
26	23	GM3	1481	15
27			1497	
28				
29				
30				

6. Save the changes. The new lipid class will appear in Start sheet (scroll button) automatically.

7. Process data in the same way, as described above.